

nonphotochemical quenching (NPQ), in which excess absorbed light is dissipated safely as heat. The primary, rapidly reversible component of NPQ is energy-dependent quenching (qE), which is induced by a low pH in the thylakoid lumen. Measuring the change in chlorophyll excited state lifetime in PSII due to specific physical processes involved in qE has been difficult because of multiple NPQ components and the difficulty of measuring ultrafast processes on a sample whose photophysical decay pathways change in response to actinic light. Using time-resolved fluorescence, we measured the lifetime of excited chlorophylls in PSII of live cells of the green alga *Chlamydomonas reinhardtii* as they adapted to variable light conditions. The average trajectory to a qE site, τ_{qE} , was measured to be 276 ps for any quenched PSII in wild-type cells. This result provides direct evidence that PSII has two primary conformations - unquenched and quenched - and that it switches between these two states based on the pH of the lumen. The appearance of the fast lifetime had a 200 ms delay and 400 ms rise time. We attribute these timescales to changes in the lumen pH, with the first timescale being the time for protons to begin building up in the lumen and the second timescale being the time for the lumen pH to drop to the pK_a of the pH sensing residues of PSII. We anticipate that these methods will be useful for probing the photophysical mechanism of qE quenching and using the dynamics of NPQ induction as a probe of chloroplast dynamics.

832-Pos Board B618

The Retinal Conformation of Metarhodopsin II Elucidated by Molecular Dynamics Simulations

Fuyuki Sakai, Shoji Takada.

Kyoto University, Kyoto, Japan.

Rhodopsin is a photo-activated G protein coupled receptor. In dark, rhodopsin consists of apoprotein, opsin, and chromophore, 11-*cis* retinal. After photo absorption, 11-*cis* retinal is isomerized to all-*trans* retinal. Then, the proton of Schiff base transfers to its counter ion and transmembrane helix (TM) 6 moves. In this state, rhodopsin can activate the G protein, transducin, and is called Metarhodopsin II (Meta II).

Activation mechanism of rhodopsin has been focused but the structure of Meta II had not been solved before 2011. In 2011, two crystal structures of active rhodopsin model were solved. In one model, Choe model, the retinal is rotated against that of Lumirhodopsin (Lumi). On the other hand, in another model, Standfuss model, the retinal is not rotated. Here, we addressed which is a good model for Meta II using molecular dynamics (MD) simulations.

In a simulation from the Choe model, the retinal is stable during 100 ns, while in a simulation from the Standfuss model, the polyene chain of retinal is unstable during 100 ns and β -ionone ring of that is rotated during 500 ns. These suggest that Standfuss model is less stable than Choe model and the retinal in Meta II is rotated against that of Lumi. Next, we tried to check how the retinal conformation changes from inactive to active state. We made inactive Meta II from Lumi and performed 1 μ s simulation of inactive Meta II. The result of simulation suggests that the retinal has some sub-states, but is not rotated in the inactive Meta II.

833-Pos Board B619

Photochromism in a Flavin Binding Photoreceptor

Carmen Mandalari¹, Aba Losi¹, Stefania Abbruzzetti^{1,2}, Cristiano Viappiani^{1,2}, Wolfgang Gärtner³.

¹Università di Parma, Parma, Italy, ²NEST, Istituto Nanoscienze-CNR, Pisa, Italy, ³Max-Planck-Institute for Bioinorganic Chemistry, Mülheim a.d. Ruhr, Germany.

We have investigated the photochromic behavior of the LOV protein YtvA using steady state and time-resolved spectroscopies.

Excitation at 475 nm of the dark adapted, fluorescent species converts the chromophore to the nonfluorescent, signalling state. We show that the dark adapted species can be obtained through irradiation with near UV (360 nm) or violet (405 nm) excitation. Under steady state illumination, the spectral overlap between the two species leads to a photoequilibrium, from which the quantum yield for forward and reverse reactions can be obtained. Similar estimates for the quantum yields are obtained from the equilibrium absorption spectra of the mixture, and from the kinetics of the photoconversion.

Switching of the protein between dark and light adapted states can be used to tune fluorescence emission, observed only in the dark adapted state, and may be exploited in super-resolution microscopy applications.

References

1. The LOV2 domain of phototropin: a reversible photochromic switch. J.T.M. Kennis, I.H.M. van Stokkum, S. Crosson, M. Gauden, K. Moffat, and R. van Grondelle, J. Am. Chem. Soc., 2004, 126, 4512–4513.
2. A. Losi, A. and W. Gärtner, Old chromophores, new photoactivation paradigms, trendy applications: flavins in LOV and BLUF photoreceptors. Photochem. Photobiol., 2011, 87, 491–510.

Computational Methods I

834-Pos Board B620

Prediction of pKa Value of Zinc-Bound Water in Carbonic Anhydrase

Dian Jiao, Susan Rempe.

Sandia National Lab, Albuquerque, NM, USA.

The deprotonation of zinc-bound water in carbonic anhydrase II is the rate-limiting step in the catalytic reaction of CO₂-bicarbonate conversion. In order to understand the factors determining the pKa of the zinc bound water at the active site, quantum chemistry calculation together with continuum model is carried out. The pKa changes due to the active site mutation are well reproduced. Additionally, the structural analysis and charge/dipole examination provide evidence that the fluctuation of the active site structure and the redistribution of the electrostatics lead to the pKa shift. Also, the distinct pKa value of the same type of mutation at different site results from asymmetric ligation and different electronic environment around the zinc ion. This study not only provides insight into the molecular mechanism of the enzyme but also helps design artificial material for CO₂ sequestration.

835-Pos Board B621

Dynamic Protonation of Biomolecules in Molecular Simulation

R. Jay Mashl.

U. Illinois, Urbana, Urbana, IL, USA.

Using molecular simulation to model a biological phenomenon requires the selection of an assumed static set of ionization states that reflects the experimental conditions. We have developed a general approach to allow for these ionization states to change dynamically during the simulation. Moreover, our framework allows for the theoretical or experimental input of ionization states as the simulation proceeds.

836-Pos Board B622

Charge Burial Energetics and Protein pKa Shifts Modeled with Nonlocal Electrostatics

Jaydeep P. Bardhan¹, Peter R. Brune².

¹Rush University Medical Center, Chicago, IL, USA, ²Argonne National Lab, Argonne, IL, USA.

Continuum electrostatic theory based on the Poisson equation has proven to be a surprisingly effective simple model for understanding protein function, but it has not enjoyed equal success in every application. In particular, calculations of protein pKa shifts have seemed to require substantially different treatment than other calculations. Many groups have found, independently, that pKa calculations match experimental data much better if one adjusts the protein dielectric constant from experimental estimates (which suggest 2 to 5) up to 10 or even higher; this empirically determined parameter has been justified by various physical arguments including implicit protein flexibility. Here we study protein pKa shifts, and charge burial more generally, using numerical simulation and analytical methods to solve Hildebrandt's "structured continuum" formulation of nonlocal electrostatics. Nonlocal solvent response accounts in a simple way for the reduction in dielectric screening that arises at short length scales due to water molecules' finite size and their tendency to form semi-structured networks. We find that the resulting reduction in charge-burial penalties allows the nonlocal model to predict pKa shifts that are comparable in magnitude to shifts computed using the local model with empirically large dielectric constants.

837-Pos Board B623

A Stochastic Technique that Computes Electrostatic Solvation and Binding Free Energies in the Generalized Born Model with Exact Born Radii

Robert C. Harris, Marcia O. Fenley.

Florida State University, Tallahassee, FL, USA.

A stochastic generalized Born (GB) solver is presented whose predictions converge to those given by exact GB radii to arbitrary precision. By solving